Multiple versus single virus respiratory infections: viral load and clinical disease severity in hospitalized children

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Background Molecular testing for viral pathogens has resulted in increasing detection of multiple viruses in respiratory secretions of ill children. The clinical impact of multiple virus infections on clinical presentation and outcome is unclear.

Objectives To compare clinical characteristics and viral load between children with multiple virus versus single virus illnesses.

Patients/methods Eight hundred and ninety-three residual nasal wash samples from children treated for respiratory illness at Children's Hospital, Seattle, from September 2003 to September 2004 were evaluated by quantitative PCR for respiratory syncytial virus (RSV), human metapneumovirus (hMPV), influenza (Flu), parainfluenza, adenoviruses, and coronaviruses (CoV). Illness severity and patient characteristics were abstracted from medical charts.

Results Coinfections were identified in 103 (18%) of 566 virus-positive samples. Adenovirus was most commonly detected in coinfections (52%), followed by CoV (50%). Illnesses with a single virus had increased risk of oxygen requirement ($P = 0.02$), extended hospital stays ($P = 0.002$), and admissions to the inpatient ($P = 0.02$) or intensive care units ($P = 0.04$). For Adv and PIV-1, multiple virus illnesses had a significantly lower viral load ($\log_{10}$ copies/ml) than single virus illnesses (4·2 versus 5·6, $P = 0.007$ and 4·2 versus 6·9, $P < 0.001$, respectively). RSV, Flu-A, PIV-3, and hMPV viral loads were consistently high whether or not another virus was detected.

Conclusions Illnesses with multiple virus detections were correlated with less severe disease. The relationship between viral load and multiple virus infections was virus specific, and this may serve as a way to differentiate viruses in multiple virus infections.

Keywords Coinfection, disease severity, PCR, pediatric, respiratory virus, viral load.

Introduction

Polymerase chain reaction testing for viral pathogens has led to the detection of simultaneous multiple viruses in the setting of respiratory illness among both healthy and immunocompromised children.[1,2] The ongoing discovery of new respiratory viruses continues to provide detailed information on potential pathogens to clinicians, but the impact of simultaneous detection of multiple respiratory pathogens from a single specimen in one child is not clear. One study of viral coinfection using standard culture methods as well as serological and molecular detection methods determined increased rates of hospitalizations in children with dual respiratory virus infections. However, this study occurred before the recent discovery of some respiratory viruses including human metapneumovirus[3] and relied on relatively insensitive culture methods. The impact of multiple viruses on the severity of clinical illness is unclear.[4–14] Several studies that focused primarily on respiratory syncytial virus (RSV) and/or human metapneumovirus documented increased hospitalization and intensive care admissions[6,8,10] or more prevalent fever[14] with viral coinfections, while other reports found no association of multiple viruses with respiratory illness severity.[12,13,15,16]

To correlate clinical disease severity and viral load in children with single and multiple virus detections, we analyzed nasal wash samples and reviewed charts of children evaluated for respiratory disease in a single pediatric center

during one respiratory virus season. We compared disease severity and virus-specific viral load between illnesses with single or multiple virus detections.

Methods

Study design and population

The study population included children clinically evaluated for respiratory symptoms at Seattle Children's Hospital, Seattle, Washington, from September 2003 to September 2004 who had residual clinical nasal wash material available for analysis and medical charts available for review. The study site hospital provides both primary and tertiary care for children from birth to age 21 years throughout the Pacific Northwest of the United States. Only one sample per child was included in the analysis. Multiple samples were available for a single admission in <2% of admissions, and the earliest positive sample was used in these cases.

Laboratory data collection

Samples were stored at −70°C until evaluated using quantitative real-time PCR for adenovirus (AdV) DNA and reverse-transcription PCR for the following RNA viruses: RSV A and B subtypes, human metapneumovirus (hMPV), influenza (Flu) A and B, parainfluenza (PIV) types 1, 2, 3, and 4, and coronavirus (CoV) subtypes 229E, HKU1, NL63, OC43.[17–20] For the quantitative assays, the threshold cycles of clinical samples were compared with standard curves generated by the amplification of known numbers of DNA plasmids (AdV) or RNA transcripts containing the primer targets. Quantitative results were not available for CoV subtypes HKU1 or NL63.

Quantitative PCR results were expressed as RNA or DNA copies per ml of original sample. To ensure that negative results were not because of poor extraction or inhibition of the PCR assay, 10^5 copies/ml (1000 copies/reaction) of EXO external control, a 130 base transcript derived from jellyfish DNA,[20] were added to the sample lysis buffer during extraction. All samples with negative respiratory virus results required detection of EXO to be considered valid.

Clinical data collection

Clinical data were abstracted by medical record review using a uniform data collection form. The following variables were collected for each child: age at sample collection, gender, duration of hospitalization, admission unit, and admission and discharge International Classification of Diseases, 9th Revision (ICD-9) codes. Clinical disease correlates collected for the time period of 24 hours before to 24 hours after the nasal sample included the following: maximum temperature, maximum respiratory rate, use of bronchodilators, use of chest radiography examination, supplemental oxygen requirement, mechanical ventilation requirement, and use of antibiotics. The study was approved by the Seattle Children's Hospital Institutional Review Board.

Statistical analyses

Quantitative variables were described using mean or median, with standard deviation or range. Differences in means were tested using two-sided t tests, and non-parametric comparisons of age were tested using Wilcoxon rank sum tests. Categorical variables were described using frequency and percent and tested using chi-squared tests. ICD-9 discharge and admission codes were grouped using the Complex Chronic Condition categories previously described by Feudtner *et al.* [21] with the addition of a group for asthma (519·1, 493·0–493·9). Multivariate linear regression (for continuous outcomes) and logistic regression (for binary outcomes) were used to compare clinical correlates of disease severity between children with single virus illnesses and multiple virus illnesses. Regression analyses controlled for age and for the presence of chronic underlying conditions. The association between multiple viruses and clinical correlates was also evaluated for consistency between age groups and within children with chronic underlying conditions. To support our findings, we examined associations with clinical severity individually for each virus (RSV coinfections versus RSV alone, for example). A two-sided P value of 0·05 or lower was considered to be statistically significant. Analyses were performed using STATA version 10.1 (College Station, TX, USA).

Results

Nasal wash samples and clinical data were available for 893 children evaluated for a respiratory illness. Altogether, 776 of the samples (87%) were collected within 1 day of presentation. The majority of the children were hospitalized in the inpatient ($n = 572$, 64%) or the intensive care units ($n = 111$, 12%). An additional 23% ($n = 205$) were evaluated and discharged from the emergency room and 0·6% from the outpatient clinics ($n = 5$). Three hundred and ninety-six (44%) children had at least one underlying chronic condition as identified by ICD-9 discharge diagnosis codes. The most common underlying condition was asthma (44%) followed by cardiac conditions (9%) (Table 1). The median age in the study population was 16 months (interquartile range: 4–44 months). Children were evenly distributed among young infants experiencing their first respiratory virus season (0–5 months, $n = 257$), older infants and toddlers (6–23 months, $n = 301$), and preschool-aged and older children (24 months and older, $n = 335$).

At least one virus was detected from 566 children (63% of study population). More than one virus was detected in

Table 1. Characteristics of study population, by single or multiple virus detection

	All collected samples (*n* = 893)	No viruses detected (*n* = 327)	Single virus illnesses (*n* = 463)	Multiple virus illnesses (*n* = 103)
Male, *n* (%)	504 (56)	181 (55)	267 (58)	56 (54)
Age at illness, *n* (%)				
0 to <6 months	257 (29)	96 (29)	143 (31)	18 (17)
6 to <24 months	301 (32)	88 (27)	155 (33)	58 (56)
24 months and older	335 (38)	143 (44)	165 (36)	27 (26)
Underlying chronic conditions, *n* (%)				
Any	396 (44)	183 (56)	179 (39)	34 (33)
Asthma	164 (18)	61 (19)	81 (17)	22 (21)
Neurologic	58 (6)	28 (9)	28 (6)	2 (2)
Cardiac	79 (9)	40 (12)	34 (7)	5 (5)
Respiratory	44 (5)	25 (8)	17 (4)	2 (2)
Renal	12 (1)	4 (1)	7 (2)	1 (1)
Gastrointestinal	15 (2)	4 (1)	9 (2)	2 (2)
Hematologic	24 (3)	12 (4)	7 (2)	5 (5)*
Metabolic	6 (1)	2 (1)	4 (1)	0 (0)
Genetic	57 (6)	22 (7)	31 (7)	4 (4)
Malignancies	58 (6)	35 (11)	21 (5)	2 (2)
Sample collected within 1 day of admission, *n* (%)	776 (87)	251 (77)	428 (92)	97 (94)

*$P < 0.05$ for difference between multiple virus illnesses and single virus illnesses.

103 (18%) children, including 96 two virus infections and 7 three virus infections. Gender distribution was similar between children with single virus illnesses versus those with multiple virus illnesses. The majority of children with multiple virus illnesses were admitted to an inpatient, non-ICU ward (*n* = 64; 62%), followed by 37 children (36%) who were evaluated and discharged from the emergency room and 2 (2%) children who were admitted to the inpatient ICU. Prevalence of multiple virus illnesses was significantly associated with patient age in a non-linear fashion. Multiple viruses were more common in children aged 6–24 months (*n* = 58/301, 27%) compared with children 0–6 months of age (*n* = 18/257, 11%; $P < 0.001$) and children aged 24 months or older (*n* = 27/335, 14%; $P = 0.001$).

RSV was the most frequently detected virus (*n* = 223; 25%) (Figure 1). The most common combinations among the 96 dual viral infections were AdV/RSV (*n* = 24), RSV/CoV (*n* = 17), and AdV/Flu A (*n* = 14). CoV and AdV were most commonly detected simultaneously with other viruses (50% and 52%, respectively), while Flu A was the least likely to be detected with other viruses (20%) (Figure 1). PIV2, PIV4, and Flu B were not detected in any sample.

AdV and PIV1 viral quantities (median $\log_{10}$ copies/ml) were significantly reduced in samples from multiple virus illnesses compared with single virus illnesses, (4·2 versus
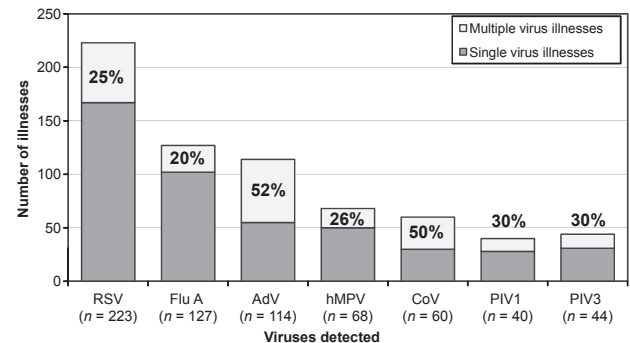

Figure 1. Percent of illnesses with multiple virus detections, by respiratory virus. RSV, respiratory syncytial virus; Flu A, influenza A; AdV, adenovirus; hMPV, human metapneumovirus; CoV, coronavirus; PIV1, parainfluenza type 1; PIV3, parainfluenza type 3.

5·6, $P = 0.007$ and 4·2 versus 6·9, $P < 0.001$, respectively) (Figure 2). In contrast, RSV, Flu A, PIV 3, and hMPV viral loads were consistently high whether or not another virus was detected. These results were generally not affected by adjustment for age and presence of chronic disease, with the exception of PIV3. In a subanalysis of children with underlying chronic conditions, PIV3 viral quantity was significantly lower in multiple virus illnesses than in single virus illnesses (5·4 versus 8·0, $P < 0.01$) in this group.
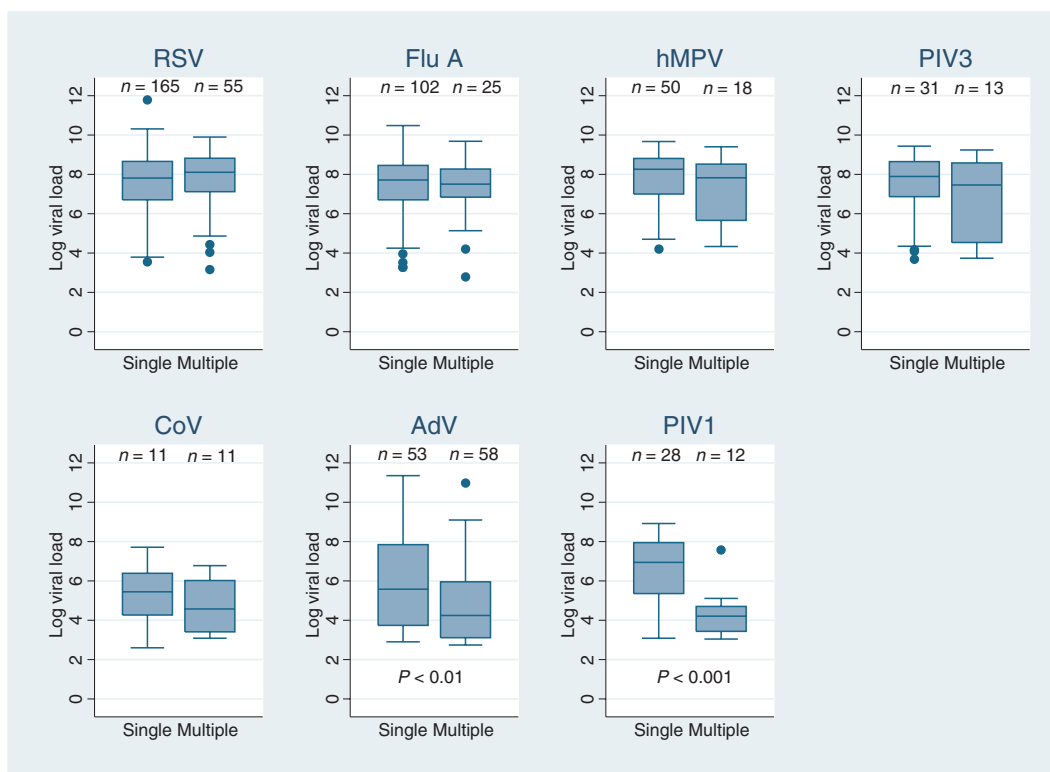
Figure 2. Distribution of respiratory virus viral loads, by single and multiple detection. Log viral load is shown separately for viruses detected in a single virus illness and those detected in a multiple virus illness. The number of positive detections for which quantitation was available for each virus is listed above each box. RSV, respiratory syncytial virus; Flu A, influenza A; AdV, adenovirus; hMPV, human metapneumovirus; CoV, coronavirus; PIV1, parainfluenza type 1; PIV3, parainfluenza type 3. [a]CoV quantitation available for subtypes 229E (*n* = 7) and OC43 (*n* = 15) only.

Children with single virus illnesses had higher rates of severe clinical disease compared with children with multiple virus infections. Children with multiple virus detections were less frequently admitted to the inpatient ward (OR = 0·55; *P* = 0·02) or to the intensive care unit (OR = 0·22; *P* = 0·04), required supplemental oxygen (OR = 0·55; *P* = 0·02), or required hospital stays longer than 3 days (OR = 0·32; *P* = 0·002) compared with the group of children with single viruses, controlling for age and the presence of an underlying chronic condition (Table 2). These reduced risks among coinfected children were consistent among all three age groups evaluated (0–5, 6–23, 24 months and older), and age group did not significantly modify the effect of coinfection on patient outcome. Among children under 2 months of age, average respiratory rate in single virus illnesses was increased by 16 breaths per minute compared with children with multiple virus illnesses (95% CI: 7, 25; *P* = 0·001). No differences were observed when comparing rates of antibiotic use, fever above 38°C, or abnormal radiographic findings. Virus-specific viral load was not associated with disease severity in this analysis.

Discussion

Our study, which assessed the presence of 10 common respiratory viruses in a cohort of children evaluated for acute respiratory disease at a single medical center, found that children with only a single virus detected were more likely to have severe illness as measured by inpatient and ICU admissions, hospital stays greater than 3 days, and need for supplemental oxygen than children who had multiple respiratory viruses detected. In contrast, we found that rates of fever and abnormal radiographic findings were similar between children with single and multiple virus illnesses. The relationships in our study between multiple virus detection and clinical disease were consistent across age groups and both healthy and chronically ill children. Notably, we found the highest prevalence of multiple virus infection among children 6–23 months of age. While younger infants (birth to 5 months of age) are undergoing exposure to their first viral season and are likely experiencing a primary infection, these older children (6–23 months) may be more likely to have had previous exposures to these respiratory viruses in an earlier season. Perhaps, the

Table 2. Correlates of severe disease, by single or multiple virus illnesses

Correlate, n (%)	All collected samples (n = 893)	Single virus illnesses (n = 463)	Multiple virus illnesses (n = 103)	OR* (95% CI; P value)	AOR** (95% CI; P value)
Admitted to inpatient ward	683 (76)	347 (75)	66 (64)	0·60 (0·38, 0·94; 0·03)	0·55 (0·34, 0·91; 0·02)
Admitted in intensive care unit	111 (12)	47 (10)	2 (2)	0·18 (0·04, 0·73; 0·02)	0·22 (0·05, 0·91; 0·04)
Extended hospital stay required (>3 days)	260 (29)	119 (26)	9 (9)	0·38 (0·14, 0·57; <0·001)	0·32 (0·15, 0·66; 0·002)
Supplemental oxygen required	291 (33)	162 (35)	23 (22)	0·53 (0·32, 0·88; 0·01)	0·55 (0·33, 0·92; 0·02)
Assisted ventilation required	91 (10)	35 (8)	1 (1)	0·12 (0·02, 0·88; 0·04)	0·13 (0·02, 1·00; 0·05)
Antibiotics used	403 (45)	201 (44)	42 (41)	0·89 (0·58, 1·38; 0·61)	0·88 (0·56, 1·37; 0·57)
Bronchodilators used	416 (47)	60 (59)	232 (50)	1·44 (0·93, 2·23; 0·10)	1·31 (0·82, 2·08; 0·24)
Abnormal radiographic finding***	202 (39)	113 (42)	22 (40)	0·90 (0·50, 1·63; 0·73)	0·87 (0·47, 1·61; 0·66)
Fever present within 24 hours of sample	346 (39)	195 (42)	45 (44)	1·07 (0·69, 1·64; 0·77)	0·95 (0·60, 1·49; 0·82)

*Crude odds ratio for multiple virus illnesses compared with single virus illnesses.
**Adjusted odds ratio, for multiple virus illnesses compared with single virus illnesses, controlling for age and presence of chronic disease.
***Among 512 chest X-rays performed (266 in single virus illness group and 55 in multiple virus illness group).

increased severity [22] and heightened immune response during a primary infection in the youngest children may discourage colonization by a second viral pathogen, leading to lowered prevalence of multiple viruses in this youngest group.

The relationship between viral load and viral coinfection differed by virus. For example, RSV, FluA, hMPV, and PIV3 were present at consistently high quantities regardless of the presence of other viruses in the sample. By contrast, the viral load for PIV 1 and AdV differed substantially depending on whether single or multiple viruses were detected. These associations may offer insight into which virus predominates in a multiple virus illness. Notably, 82% of coinfections consisted of one virus from the group with consistently high viral load (RSV, Flu A, hMPV, or PIV3) combined with an alternate virus (CoV, PIV 1, or AdV). This may suggest a possible model for virus coinfections that include one predominant virus and one virus that is present at a lower quantity and does not confer increased severity. The presence of low-quantity CoV and AdV in a coinfection merits further study, especially given the finding that the prevalences of these two viruses in asymptomatic individuals are second only to rhinovirus.[16,23,24]

Similar to our findings, Canducci et al. reported a lower prevalence of multiple virus illnesses in the youngest infants and an increased severity of disease (as measured by increased hospital stay and prevalence of hypoxia) in RSV-only illnesses compared with those with both RSV and hMPV detected.[4] However, our results are in contrast to several studies which found increased hospital admissions,[3,5] intensive care unit admissions,[9,10] and duration of hospitalization and need for supplemental oxygen,[6] or,

recently, increased presence of fever[14] for illnesses with multiple virus detections compared with those with single virus detections. Some of these differences may be attributed to variation in the age ranges studied[3,10] or the restriction to specific respiratory illnesses[5,14] or to RSV or RSV/hMPV coinfection in particular.[6,10,14] Other studies have reported no association between respiratory illness severity and multiple virus detections,[7,12,13] including very recent reports.[15,16]

It is, perhaps, counterintuitive that the presence of multiple species at a single time point is not associated with more severe disease. An immune response to an infection with the first virus could modify the disease severity of a subsequently acquired virus, potentially by the induction of interferon and other anti-viral response modifiers. While our sample size was too small to determine whether our results reflect the number of viruses detected or the characteristics of specific virus combinations, we found our estimates to be largely consistent when stratified by virus, although not statistically significant.

Although our study is among the largest to evaluate this question using clinical data so closely tied to the time of virus testing (within 24 hours), there are several study limitations. Our study design precluded identifying incident viral infections, limiting our ability to define acquisition of each virus in relation to symptom onset. Similarly, the course of the illness prior to presentation and collection of the respiratory specimen could not be reliably verified. Although viral load generally decreases from illness onset,[25] our study was able to compare viral loads at the time of illness symptoms that warranted medical attention. The total number of viral coinfections was likely underestimated

because we did not evaluate the presence of rhinovirus and bocavirus, viruses known to be associated with prolonged viral shedding and detection during both symptomatic and asymptomatic periods.[24,26,27] Thus, some cases of single infection in our study could be classified as multiple infections in studies which include these viruses. We were unable to determine rhinovirus viral load because of the large number of serotypes present, making it difficult to differentiate asymptomatic shedding from active infection. We believe these particular pathogens are better studied in prospective settings where baseline viral shedding can be examined.[24] We also did not assess potential bacterial pathogens, although the comparable rates of antibiotic use between the two study groups (Table 2) indicated that suspected or documented bacterial infection is an unlikely confounder.

In our study population of children evaluated for acute respiratory infection, we observed that multiple virus combinations of RSV, hMPV, PIV, Flu A, and AdV were more common in children 6–24 months of age. Lessened illness severity was observed among multiple virus illnesses. We determined the relationship between viral quantity and co-infections to be virus specific, and we hypothesize that viral load may serve as an important clue as to which virus in a mixed infection may have a greater influence on the clinical severity of the illness.

Finding/Support

Acknowledgements

Conflicts of interest

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